

# Utilization of Crop Residues and Solubilizing Bacteria to Enhance the Dissolution of Moroccan Phosphate Rock

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## ABSTRACT

Phosphorus is essential for plant growth, but its availability in soils is limited due to low solubility of phosphate compounds. This study evaluated the combined effects of crop residues (CR) and phosphate-solubilizing bacteria (PSB) on the dissolution of Moroccan phosphate rock. Pot experiments were conducted for 60 days using different doses of MPR (450 kg/ha, 300 kg/ha, and 150 kg/ha), rice and maize crop residues, and a PSB consortium from a rice paddy field. Parameters such as pH, bacterial count, and phosphorus content were measured. The pH increase was significantly higher under the T3 treatment (CR + PSB) with pH 8.26 to 8.58, followed by T1 (CR alone) with pH 7.73 to 8.1, T2 (PSB alone) with pH 7.4 to 7.91, and T0 (control) with pH 7.27 to 7.67. The number of PSB was highest under the T3 treatment ( $1238.5 \times 10^6$  to  $2081.1 \times 10^6$  bacteria/g dry matter), followed by T1 ( $127.2 \times 10^6$  to  $249.03 \times 10^6$  bacteria/g dry matter) and T2 with  $1.7 \times 10^6$  to  $44.6 \times 10^6$  bacteria/g dry matter, regardless of the MPR dose. Phosphorus solubilization was 35% to 62% with PSB alone (T2), 72% to 88.5% with CR alone (T1), and over 83.98% when both CR and PSB were combined (T3). This increase was more pronounced with higher MPR doses (300 to 450 kg/ha). Incorporating PSB and crop residues as biofertilizers is a viable strategy for enhancing phosphate rock dissolution and improving soil fertility in agricultural systems.

**Keywords:** Bacteria number; P content; pH medium; Solubilization; Phosphate reducing bacteria; Moroccan phosphate rock; Biophosphocompost.

## 1. Introduction

Phosphorus (P) is a critical nutrient for plant growth and development, after to nitrogen (Anand et al., 2016; Amri et al., 2023; Guety et al., 2024). It is an essential component of all major metabolic pathways in plants, including photosynthesis, signal transduction, and respiration (Sharma et al., 2013). However, the concentration of soluble P in soil is often limited for its low solubility ( $400\text{--}1260 \text{ mg kg}^{-1}$ ) (Amri et al., 2023). This necessitates the use of fertilizers to ensure higher crop productivity, responding to the increasing demand driven by the continuous growth of the world population (Bindraban et al., 2020). The major challenge with the application of chemical fertilizers is that a large portion of the soluble form of inorganic phosphate applied to the soil is rapidly immobilized and becomes unavailable to plants (Kumar et al., 2017). Additionally, higher availability of P in the soil can reduce the assimilation of heavy metals, such as cadmium, thereby mitigating the risk of negative effects on plant growth as well as animal and human health through the food chain (Azzi et al., 2017). In this context, biofertilizers emerge as a promising solution, enabling the maintenance of soil fertility while mitigating the environmental impact associated with chemical fertilizers (Biaou et al., 2017; Gnahoua et al., 2023; Agegnehu et al., 2021). Consequently, the search for innovative strategies to enhance soil fertility has become a priority. This includes the application of amendments such as natural phosphate rock (Saleem et al., 2013; Mashori et al., 2013; Abbasi et al., 2015), the utilization of soil microorganisms as biofertilizing agents (Asuming-Brempong and Anipa, 2014; Taktek, 2015), and the valorization of crop residues and organic waste, providing a sustainable solution for their disposal (Dagbenonbakin et al., 2013).

Phosphate-solubilizing microorganisms are regarded as typical plant growth-promoting rhizobacteria (PGPR) that can transform insoluble phosphate into soluble form and promote plant growth (Bakki et al., 2017; Khan et al.,

2007; Tariq and Ambreen, 2023). Among phosphorus-solubilizing microorganisms, Phosphate-solubilizing bacteria (PSB) are predominant and play an important role in the biogeochemical phosphorus cycling in both terrestrial and aquatic environments (Qingwei et al., 2023; Tariq and Ambreen, 2023). These bacteria can solubilize insoluble phosphate compounds, thereby improving phosphate availability in soil (Bakki et al., 2017; Tariq and Ambreen, 2023). The use of PSB not only enhances plant growth by increasing phosphate uptake but also offers an environmentally friendly alternative to chemical fertilizers, supporting sustainable agricultural practices (Tariq and Ambreen, 2023).

Crop residues, primarily lignocellulosic biomass rich in carbon, play a key role in nutrient recycling (Agegnehu et al., 2021; Coulibaly et al., 2020; Fu et al., 2021; Singh and Rengel, 2007). Returning crop residues to the soil is crucial as they serve as a primary source of carbon and soil organic matter in agricultural systems, significantly influencing nutrient retention, soil structure, and water holding capacity (Agegnehu et al., 2021; Coulibaly et al., 2020; Fu et al., 2021; Singh and Rengel, 2007). Their use in agriculture enhances nutrient availability, limits soil runoff and erosion, and increases water retention, essential for plant and microorganism development (Kumari et al., 2019). Conversely, burning crop residues diminishes soil organic matter and impairs the soil's capacity to retain essential nutrients like nitrogen (N), phosphorus (P), and potassium (K), as well as its ability to hold plant-available water (Yadav et al., 2020).

Effective management of crop residues is vital for sustainable agriculture and enhancing soil health. Exploring their synergistic use with phosphate-solubilizing bacteria can positively influence soil microbial communities involved in crop residue decomposition (Shamshitov et al., 2024) and improve rock phosphate dissolution (Guety et al., 2024), contributing to more efficient and sustainable agricultural practices. When mixed in compost, crop residues can enhance the dissolution of phosphate rock, further boosting phosphorus availability.

### 1.1. Study Objectives

The objective of this study was to evaluate the combined effects of crop residues and phosphate-solubilizing bacterial consortium on the dissolution of Moroccan phosphate rock and to determine the best dose of phosphate rock added in the compost to improve phosphorus nutrition. Thus, in this context, different doses of phosphate rock were combined with crop residues and phosphate-solubilizing bacterial consortium was study in batch at laboratory to assess their impact on the pH culture medium, phosphorus P content, number of bacteria, cinetic of dissolution of Moroccan phosphate rock.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Plant Materials

The compost mixture is constituted of crop residues (CR) and poultry manure. The crop residues include rice and maize straw. Poultry manure is composed of droppings from broiler and layer chickens. The chemical characteristics of these materials are detailed in Table 1 (Boueunan et al., 2024). The analysis of crop residues reveals they are primarily composed of carbon (49.3%), potassium (25.0 g/kg), nitrogen (19.6 g/kg), phosphorus

(1.03 g/kg), calcium (2.9 g/kg), and magnesium (1.93 g/kg). They also contain trace elements such as iron (4.66 g/kg) and zinc (5.7 g/kg).

The poultry manure used in this study is characterized by a high organic matter content, with nitrogen at 3.1% and carbon at 42.1%, resulting in a C/N ratio of 13.37, indicating good decomposition potential. The manure is moderately rich in phosphorus (1.14%), potassium (2.8%), calcium (4.09%), and magnesium (1.36%), but it is relatively low in trace elements, with iron at 1.06 g/kg and zinc at 0.03 (Table 1).

**Table 1.** Chemical characteristics of Crop Residues (CR) and poultry Manure Used in the Compost Mixture

Nutrients	Values (g.kg <sup>-1</sup> )	
	Crop residues (CR)	Poultry manure
N (g/kg)	19.60	30.94
P (g/kg)	1.03	11.48
K (g/kg)	25.00	28.84
Ca (g/kg)	2.9	40.96
Mg (g/kg)	1.93	13.63
Fe	4.66	1.06
Zn	5.7	0.03
C (g/kg)	493.3	420.90
C/N	25.16	13.37
MO (g/kg)	986.6	841.8

### 2.1.2. Rock Phosphate

The phosphate rock from Morocco (PRM) was provided by OCP-Africa (Office Chérifien of Phosphate). Its chemical composition is detailed in Table 2.

**Table 2.** Chemical composition of phosphate rock in Morocco

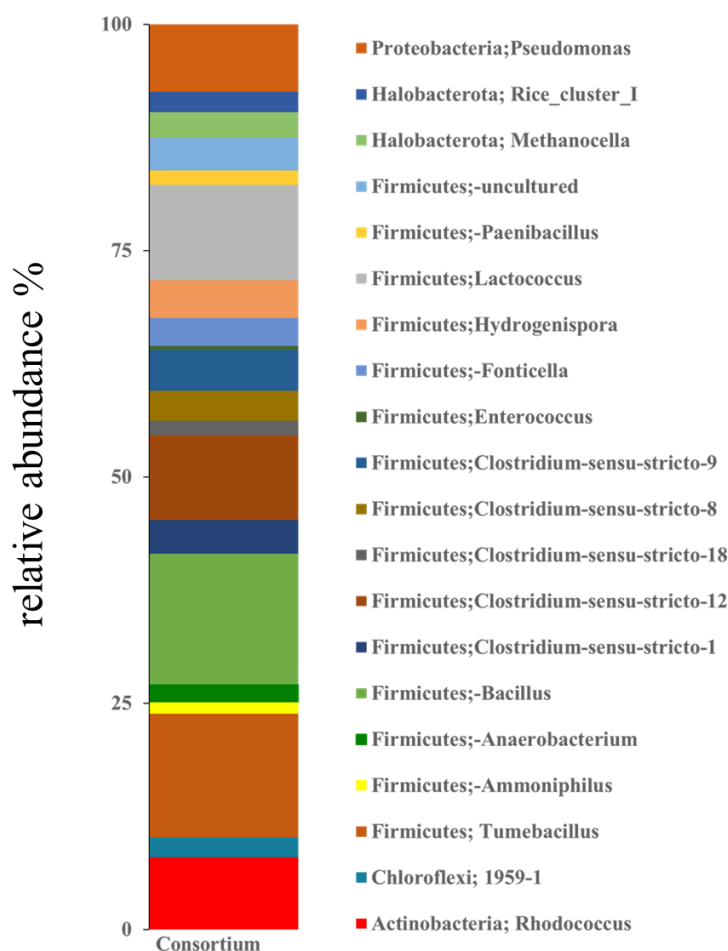
Chemical elements	P <sub>2</sub> O <sub>5</sub>	CO <sub>2</sub>	SO <sub>3</sub>	SiO <sub>2</sub>	CaO	MgO	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	F <sub>2</sub> O	H <sub>2</sub> O
Content (%)	30	6.44	1.29	6.64	49.54	1.16	0.2	0.41	2.21	2.13

### 2.1.3. Biological Materials

A phosphate-solubilizing bacterial consortium, isolated from rice paddies soil at Man (Ivory Coast) and whose microbiological characteristics are presented in Figure 1, was used to prepare the inoculum solution according to the procedure described by Boueunan et al. (2024).

Five (05) phyla were identified in the consortium. The most dominant is the phylum Firmicutes (77%), followed by the phyla Actinobacteria (8%) and Proteobacteria (8%), and finally the phyla Halobacterota (5%) and Chloroflexi

(2%) (Figure 1). In the consortium, the most representative genera in the phylum Firmicutes are *Clostridium* sp. (22.5%) and *Bacillus* sp. (14.5%) (Figure 1).



**Figure 1.** Relative proportions of the main prokaryotic genera detected in the consortium

## 2.2. Methods

### 2.2.1. Experimental Setup

To study the impact of interactions between crop residues (CR) and phosphate-solubilizing bacteria (PSB) on the solubilization of Moroccan phosphate rock (MPR), a pot experiment was conducted in the laboratory. In 800 cm<sup>2</sup> pots, 6 g of crop residues were introduced as a carbon source to simulate 750 kg of residues per hectare. Subsequently, three different doses of phosphate rock: 1.2 g MPR per pot, 2.4 g MPR per pot, and 3.6 g MPR per pot, corresponding to 150 kg MPR per hectare, 300 kg MPR per hectare, and 450 kg MPR per hectare, respectively were added to each pot. Poultry manure, representing one-third of the weight of the crop residues (CR) was also added to each pot. Finally, 600 ml of sterilized distilled water were added to each pot to achieve an 80% moisture level, and the contents were homogenized. For the inoculated treatments, each pot received 250 µl of a previously prepared inoculum solution, amounting to 10<sup>8</sup> bacteria per pot. The pots were then shaken and incubated for sixty days. After 0, 10, 20, 30, 40, 50, and 60 days of incubation, 5 ml samples of the different solutions were taken from each pot, centrifuged at 4000 rpm for 15 minutes, and the supernatant was used to measure the pH of the medium and to determine the phosphorus content by colorimetry using a spectrophotometer at 790 nm. Phosphorus in

solution is determined by spectrophotometry using the Spectroquant Phosphate Kit, which is a method based on the quantification of orthophosphate ions. Absorbance is measured at 790 nm and converted to mg P/l using a previously established standard curve. The bacterial count was also determined using a spectrophotometer by measuring the optical density at 620 nm.

The experimental setup included crop residues (CR), phosphate-solubilizing bacteria (PSB), and varying doses of MPR. A total of twelve treatments were applied, resulting from the combination of the three doses of MPR (D150, D300, D450) and the presence or absence of CR and/or PSB:

- T0D150: 150 kg/ha MPR without CR and PSB
- T0D300: 300 kg/ha MPR without CR and PSB
- T0D450: 450 kg/ha MPR without CR and PSB
- T1D150: 150 kg/ha MPR with CR, without PSB
- T1D300: 300 kg/ha MPR with CR, without PSB
- T1D450: 450 kg/ha MPR with CR, without PSB
- T2D150: 150 kg/ha MPR without CR, with PSB
- T2D300: 300 kg/ha MPR without CR, with PSB
- T2D450: 450 kg/ha MPR without CR, with PSB
- T3D150: 150 kg/ha MPR with CR and PSB
- T3D300: 300 kg/ha MPR with CR and PSB
- T3D450: 450 kg/ha MPR with CR and PSB

### 3. Results

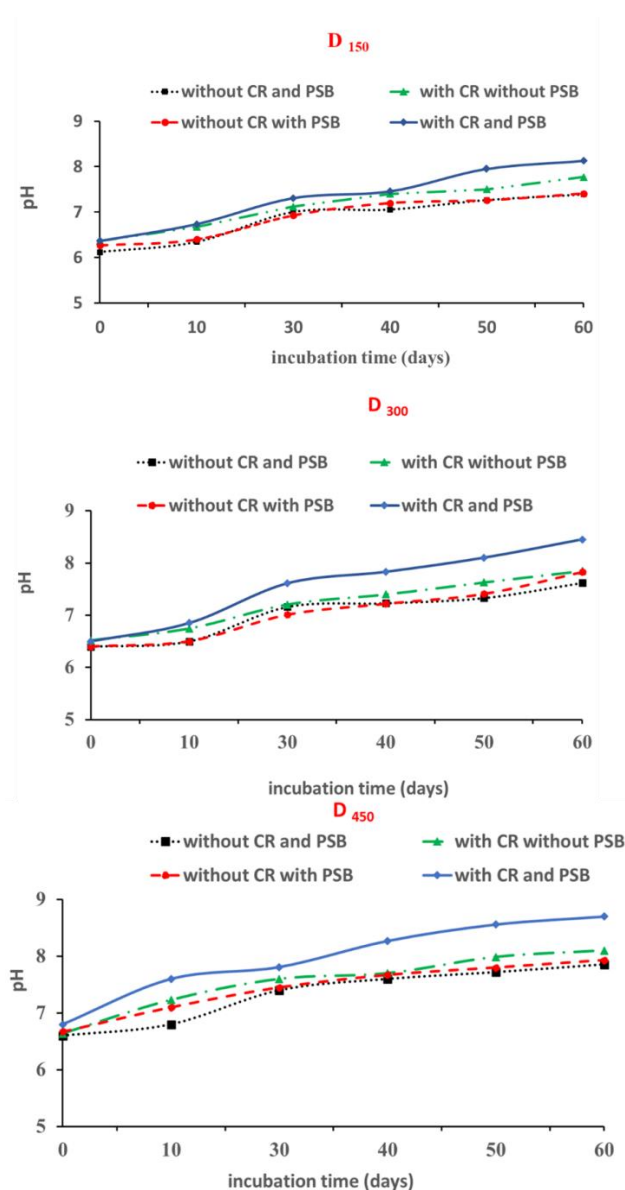
#### 3.1. pH changes in culture medium

Under all treatments, there was a highly significant increase ( $P \leq 0.0001$ ) in the pH of the medium over the incubation period in the presence of crop residues (CR) alone (T1), phosphate-solubilizing bacteria (PSB) alone (T2), and in the presence of CR combined with PSB (T3), compared to the control treatment T0, regardless of the dose of MPR applied (Figure 2). Notably, in the presence of both crop residues (CR) and PSB, the pH of the medium increased significantly faster, reaching 8.26 at D150, 8.41 at D300, and 8.6 at D450. This was in contrast to the control treatments (-CR-PSB), which ranged from 7.27 to 7.67, and the T1 (7.73 to 8.1) and T2 (7.4 to 7.83) treatments, regardless of the MPR dose (Table 3). Overall, the pH increase was significantly higher under the T3 treatment (pH 8.26 to 8.58), followed by T1 (7.73 to 8.1), T2 (7.4 to 7.91), and T0 (7.27 to 7.67). Regardless of the treatments applied, there was a significant increase in the pH of the medium when the MPR dose was high, with an average pH of 8.06 under D450 compared to the D150 dose (with an average pH of 7.67) and the D300 dose (with an average pH of 7.92) (Table 3). Increasing the dose of Moroccan phosphate rock (MPR) in the medium significantly elevated the pH by 0.25 to 0.39 units compared to D150 (Table 3).

**Table 3.** Average pH in medium after 60 days of incubation

Treatments	Doses		
	D150	D300	D450
T0 (-CR-PSB)	7.27 ± 0.07d	7.6 ± 0.08c	7.67 ± 0.2d
T1 (+CR-PSB)	7.73 ± 0.07b	7.85 ± 0.1b	8.10 ± 0.1b
T2 (-CR+PSB)	7.40 ± 0.01c	7.83 ± 0.02b	7.91 ± 0.04c
T3 (+CR+PSB)	8.26 ± 0.1a	8.41 ± 0.2a	8.58 ± 0.2a
Mean	7.67	7.92	8.06
CV (%)	1.03	1.35	1.72
Pr>F	<0.0001	<0.0001	<0.0001

In the same column, values with the same letter are not significantly different according to the Newman-keuls test  $p < 0.05$ .



**Figure 2.** Evolution of pH medium during incubation time under different treatments: T0 (without crop residues (CR) and phosphate solubilizing bacteria (PSB)); T1 (with crop residues (CR) without phosphate solubilizing bacteria (PSB)); T2 (without crop residues (CR) with phosphate solubilizing bacteria (PSB)); T3 (with crop residues (CR) and phosphate solubilizing bacteria (PSB)).

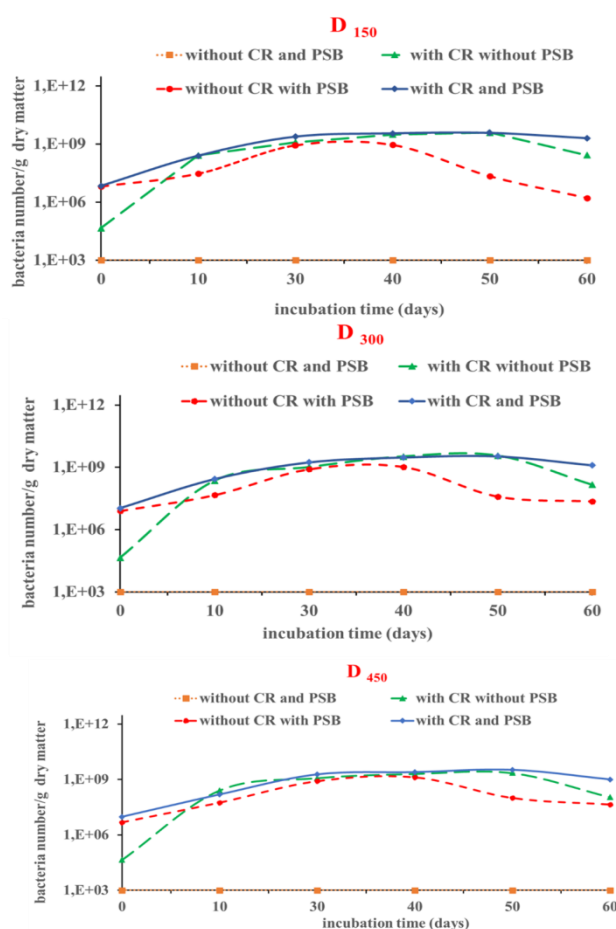
### 3.2. Phosphate-solubilizing bacteria (PSB) number Changes

The evolution of phosphate-solubilizing bacteria (PSB) in the medium during the incubation period indicates a highly significant increase ( $P \leq 0.0001$ ) in the number of PSB in the presence of crop residues (CR) alone (T1), PSB alone (T2), and CR combined with PSB (T3), compared to the control treatment (T0), regardless of the Moroccan Phosphate Rock (MPR) dose in the medium (Figure 3).

**Table 4.** Average phosphate solubilizing bacteria number per dry matter in medium after 60 days of incubation

Treatments	Dose		
	D150	D300	D450
T0 (-CR-PSB)	0 d	0 d	0 d
T1 (+CR-PSB)	$249,03 \pm 16,9$ b	$153,76 \pm 42,6$ b	$127,2 \pm 21,4$ b
T2 (-CR+PSB)	$1,7 \pm 0,2$ c	$23,5 \pm 1,3$ c	$44,6 \pm 3,7$ c
T3 (+CR+PSB)	$2081,1 \pm 231,2$ a	$1336,3 \pm 265,8$ a	$1238,5 \pm 203,3$ a
Mean	582,96	378,4	352,6
CV (%)	18,41	32,94	26,84
Pr>F	<0,0001	<0,0001	<0,0001

In the same column, values with the same letter are not significantly different according to the Newman-keuls test  $p < 0.05$ .



**Figure 3.** Evolution of phosphate solubilizing bacteria number per dry matter during incubation time under different treatments: T0 (without crop residues (CR) and phosphate solubilizing bacteria (PSB)); T1 (with crop residues (CR) without phosphate solubilizing bacteria (PSB)); T2 (without crop residues (CR) with phosphate solubilizing bacteria (PSB)); T3 (with crop residues (CR) and phosphate solubilizing bacteria (PSB)).

This number is significantly higher under the T3 treatment (CR + PSB), ranging from  $1238.5 \times 10^{-6}$  to  $2081.1 \times 10^{-6}$  bacteria/g dry matter, followed by the T1 treatment (CR - PSB) with  $127.2 \times 10^{-6}$  to  $249.03 \times 10^{-6}$  bacteria/g dry matter, and the T2 treatment (PSB - CR) with  $1.7 \times 10^{-6}$  to  $44.6 \times 10^{-6}$  bacteria/g dry matter, regardless of the MPR dose (Table 4). However, our results show a higher average number of PSB at the D150 dose, with  $582.96 \times 10^{-6}$  bacteria/g dry matter, compared to  $378.4 \times 10^{-6}$  bacteria/g dry matter at the D300 dose, and  $352.6 \times 10^{-6}$  bacteria/g dry matter at the D450 dose (Table 4). There is a significant ( $p \leq 0.05$ ) decrease in PSB number as the MPR dose in the medium increases (Table 4).

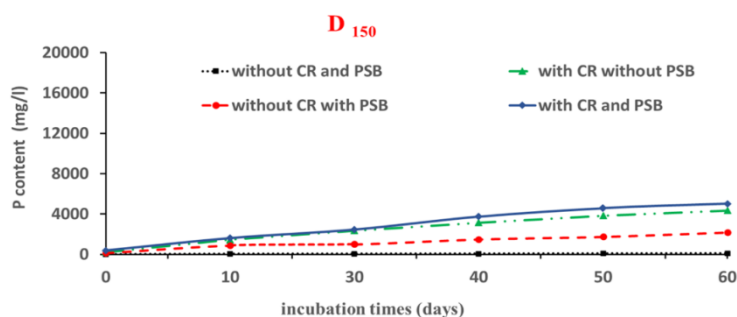
### 3.3. Phosphorus Content in culture medium

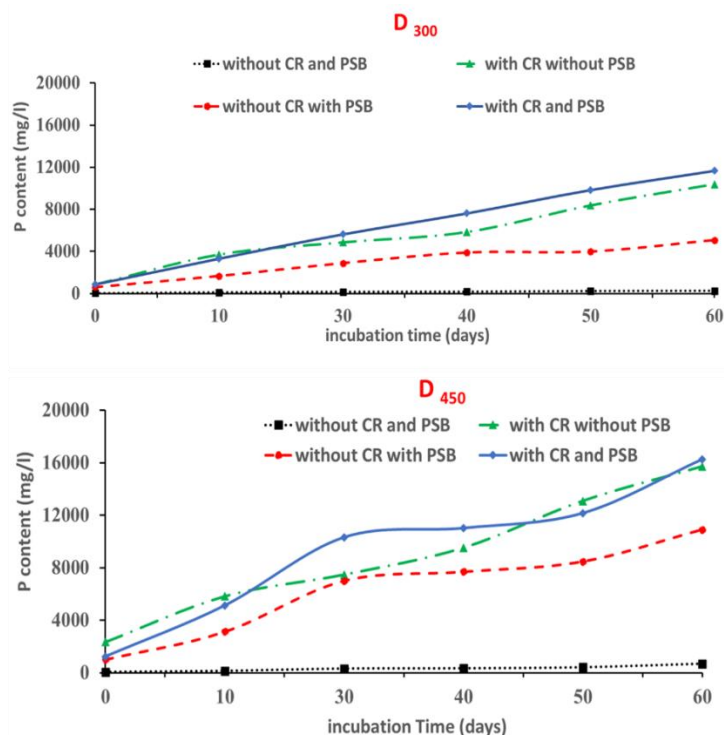
The evolution of solubilized phosphorus indicated that regardless of the dose of Moroccan Phosphate Rock (MPR) applied, there is a very significant increase during incubation time ( $P \leq 0.0001$ ) of P content in the medium in the presence of crop residues (CR) alone (T1), phosphate-solubilizing bacteria (PSB) alone (T2), and also in the presence of CR combined with PSB (T3), compared to the control treatment (T0) (Figure 4). However, this P content is significantly higher ( $P \leq 0.0001$ ) when the medium contains both crop residues and PSB, with more than 83.98% of P solubilized compared to the initial P content, regardless of the MPR dose applied (Table 5), compared to the control (1.76% to 3.86% of P solubilized compared to the initial P content). In the presence of PSB alone (T2), 35% to 62% of P is solubilized, whereas in the presence of CR alone (T1), 72% to 88.5% of P is solubilized compared to the initial P content (Table 5). Additionally, regardless of the treatment applied, there is a more significant solubilization of MPR at the D450 dose (60.82% P solubilized compared to the initial P content,  $P \leq 0.0001$ ) followed by D150 dose (48.56% P solubilized compared to the initial P content) and D300 dose (57.67% P solubilized compared to the initial P content) (Table 5).

**Table 5.** Quantité de P solubilisés (%) par rapport à la quantité initiale apportée après 60 jours d'incubation

Treatments	Dose		
	D150	D300	D450
T0 (-CR-PSB)	$1,76 \pm 0,03$ d	$2,15 \pm 0,1$ d	$3,86 \pm 0,2$ c
T1 (+CR-PSB)	$72,55 \pm 2,1$ b	$88,53 \pm 2,03$ b	$88,5 \pm 6,8$ a
T2 (-CR+PSB)	$35,98 \pm 0,3$ c	$43,2 \pm 1,4$ c	$62,15 \pm 6,1$ b
T3 (+CR+PSB)	$83,98 \pm 1,3$ a	$96,84 \pm 1,6$ a	$88,78 \pm 4,3$ a
Mean	48,5	57,67	60,82
CV (%)	2,37	2,33	7,67
Pr>F	<0,0001	<0,0001	<0,0001

In the same column, values with the same letter are not significantly different according to the Newman-keuls test  $p < 0.05$ .





**Figure 4.** Evolution of P content (mg/l) during incubation time under different treatments: T0 (without crop residues (CR) and phosphate solubilizing bacteria (PSB)); T1 (with crop residues (CR) without phosphate solubilizing bacteria (PSB)); T2 (without crop residues (CR) with phosphate solubilizing bacteria (PSB)); T3 (with crop residues (CR) and phosphate solubilizing bacteria (PSB)).

#### 4. Discussion

The study on the impact of crop residues (CR) and/or phosphate-solubilizing bacteria (PSB) on the dissolution of Moroccan Phosphate Rock (MPR) revealed a highly significant increase ( $P=0.001$ ) in P content, pH, and the number of PSB when CR is combined with PSB, compared to CR alone or PSB alone. This increase in pH and P content is more pronounced when the MPR dose is high (300 to 450 kg/ha). According to Dabre et al. (2017), this pronounced pH increase in treatments with high MPR doses (D450 and D300) may be linked to the nature of MPR, which contains 49.5% CaO.

During MPR dissolution,  $\text{Ca}^{2+}$  ions interact with  $\text{H}^+$  ions in the solution, reducing the  $\text{H}^+$  ion concentration in the soil solution and consequently increasing the pH of the medium. Furthermore, the higher P solubilization rate observed at the D300 or D450 doses is related to the  $\text{P}_2\text{O}_5$  content of MPR, which varies with each dose. Our results showed low proliferation of PSB when they were associated solely with phosphate rock (T2). This decrease in the number of PSB during the incubation period could be due to the lack of a carbon source in the medium, inhibiting their proliferation as demonstrated by Guety et al. (2024).

It was also observed that treatments with crop residues alone (T1) resulted in an increase in P content, the number of PSB, and the pH of the medium. This increase in the number of PSB could be attributed to the microorganisms present in the poultry manure used, indicating the presence of microorganisms involved in P cycling. Therefore, the presence of an organic source (crop residues) in the medium could be utilized by microorganisms for their specific activities.

The rise in pH could be attributed to the mineralization of nitrogen into ammoniacal form during the degradation processes of crop residues and the release of bases previously integrated into the organic matter (Chennaoui et al., 2016; Biekre et al., 2018), as well as the calcium present in the MPR. These processes indirectly influence the dissolution of MPR. According to Plassard et al. (2015) and Guety et al. (2024), the addition of organic matter (rice and maize straw) as a carbon source in the medium stimulates the mineralization of P contained in the MPR and also in the straw, increasing the P content in the medium (Zheng et al., 2019).

Similarly, the combination of PSB with crop residues in the dissolution of MPR yields much better results in terms of the proliferation of phosphate-solubilizing bacteria and the dissolution of MPR. This result indicates that the presence of PSB in the medium accelerates the degradation of organic residues (rice and maize straw), which are used as a carbon source to directly dissolve the MPR as demonstrated by Boueunan et al., (2024). These findings justify the use of a biofertilizer to enhance the dissolution of natural phosphate rocks, as suggested in numerous studies (Asuming-Brempong and Anipa, 2014; Tariq and Ambreen 2023; Taktek, 2015).

This study reveals that the higher the dose of MPR in the medium, the greater the increase in pH and P content during incubation. This result corroborates the findings of Kpan (2018), who observed that treatments with 100% MPR yielded higher pH and P levels compared to those containing 10% MPR.

## **5. Conclusion**

Our study revealed that the combined approach of using phosphate-solubilizing bacteria (PSB) with crop residues (CR) not only increases phosphorus (P) content but also raises pH and boosts PSB proliferation in the soil. These results strongly support the use of biofertilizers that incorporate both crop residues and PSB to improve soil fertility. This approach aligns with sustainable agricultural practices by utilizing natural processes to enhance nutrient availability, thus promoting healthier and more productive soils. Consequently, incorporating PSB and crop residues as biofertilizers presents a viable strategy for improving the efficiency of phosphate rock dissolution and enhancing soil fertility in agricultural systems.

### **Declarations**

#### **Source of Funding**

No funding source is reported for this study.

#### **Conflicts of interest**

The authors declare no conflicts of interest.

#### **Authors' Contributions**

Affi Jeanne BONGOUA-DEVISME and Wongbe Beralex BOUEUNAN contributed to the fieldwork, design, writing, and formatting of the article. YOBOUE Kouadio Emile and Franck Michael Lemounou BAHAN supervised all stages of this work.

#### **Consent for Publication**

The authors declare that they consented to the publication of this study.

### Ethical Approval

The authors stated that the study does not require ethical approval as it does not deal with humans or animals neither does it directly affect human social life.

### Availability of data and materials

Data supporting the findings and conclusions are available upon request from corresponding author.

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